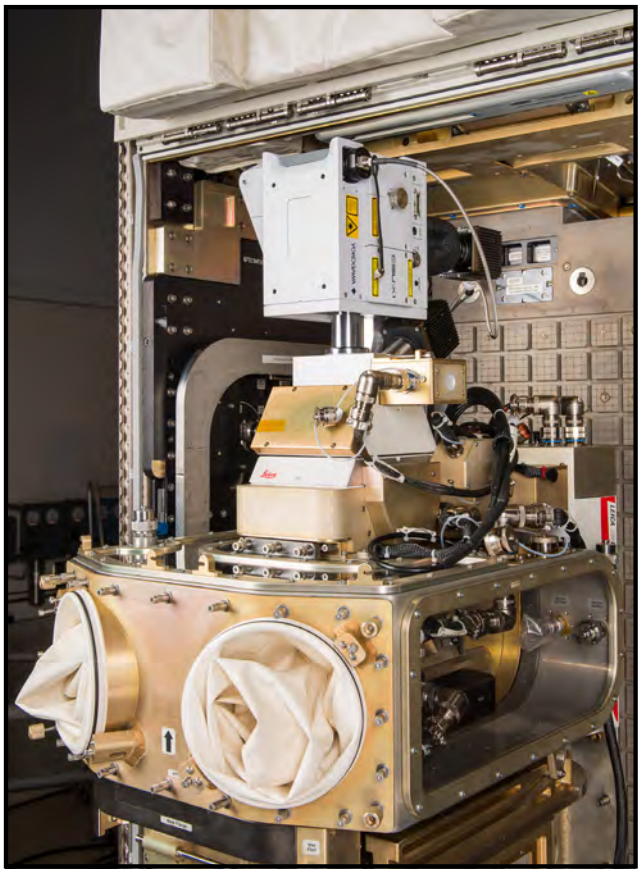
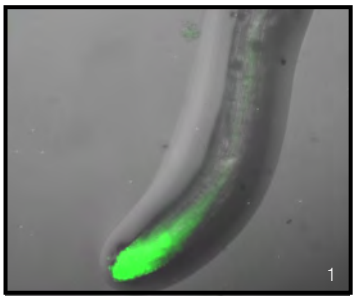
National Aeronautics and
Space Administration

Light Microscopy Module



International Space Station
Premier Automated Microscope

Space Life and Physical Sciences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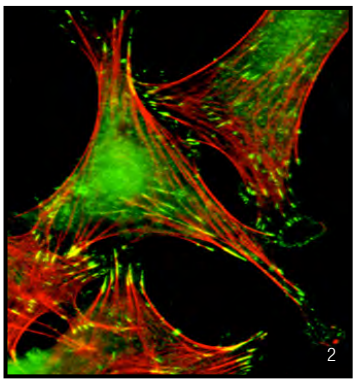
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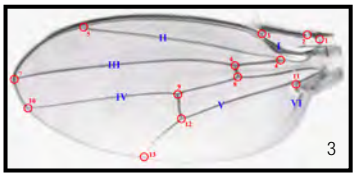
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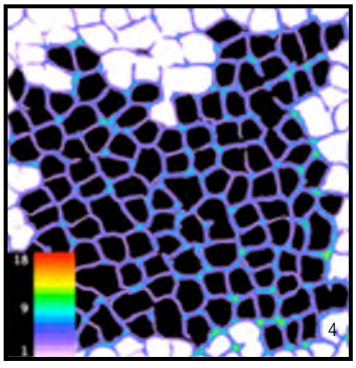
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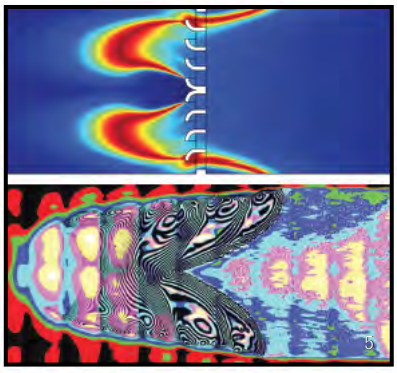
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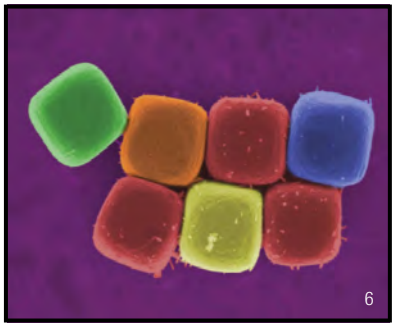
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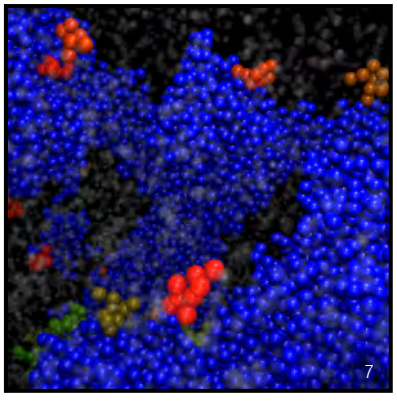
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1. Fluorescently tagged root of Arabidopsis thaliana (thale cress) from Characterizing Arabidopsis Root Attractions (CARA, ISS SpaceX-3), provided by Anna-Lisa Paul and Rob Ferl, University of Florida Gainesville.
2. Fluorescently labeled actin (red) and focal adhesion (green) in rabbit synovial fibroblasts, provided by Eduardo Almeida, NASA Ames Research Center.
3. Wing of Drosophila melanogaster (fruitfly), from Joseph Kunkel and Brian Bettencourt, http://bcrc.bio.umass.edu/flyclub/kunkel/kb_preview/index.html (Open Source).
4. Mouse vasculature by Chen, Reinecker, Parsons–Wingerter et al. from PLoS ONE.
5. Composite of CVB data, Cover–Transport Phenomena Fundamentals, 3rd Edition, Joe Plawsky.
6. Supercubes, Cover–Soft Matter, July 2011, Rossi, Sacanna, Irvine, Chaikin, Pine, Phillipse.
7. Three-dimensional model of colloidal gelation, Peter Lu, Harvard, PeterLu.org.

An Advanced Automated Microscope

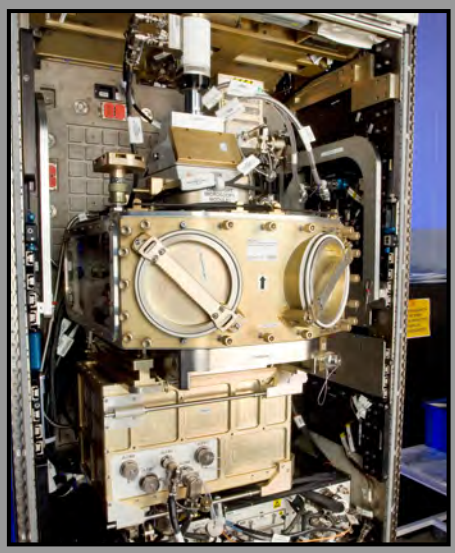
Innovative Microscopy Research Capability from Space

Innovation and imagination are all that are required to use the Light Microscopy Module (LMM) as a laboratory microscope to perform research aboard the International Space Station (ISS). The LMM is a remotely controllable, automated microscope that gives scientists the ability to study—in real time—the effects of the space environment on physics and biology. Specimens can be studied without the need to return the samples to Earth.

Microscope Modified for Space Research

The LMM flight unit features a modified commercial laboratory Leica RXA microscope configured to operate in an automated mode with interaction from the ground support staff. Its core capabilities include a level of containment, white light imaging (available now), fluorescence, confocal microscopy (available in 2016 to 2017), and an imaging capability from a Q-Imaging Retiga 1300 camera.

Microscope modified for space research.



LMM mounted in the FIR rack.

Assembly of LMM on the ISS.

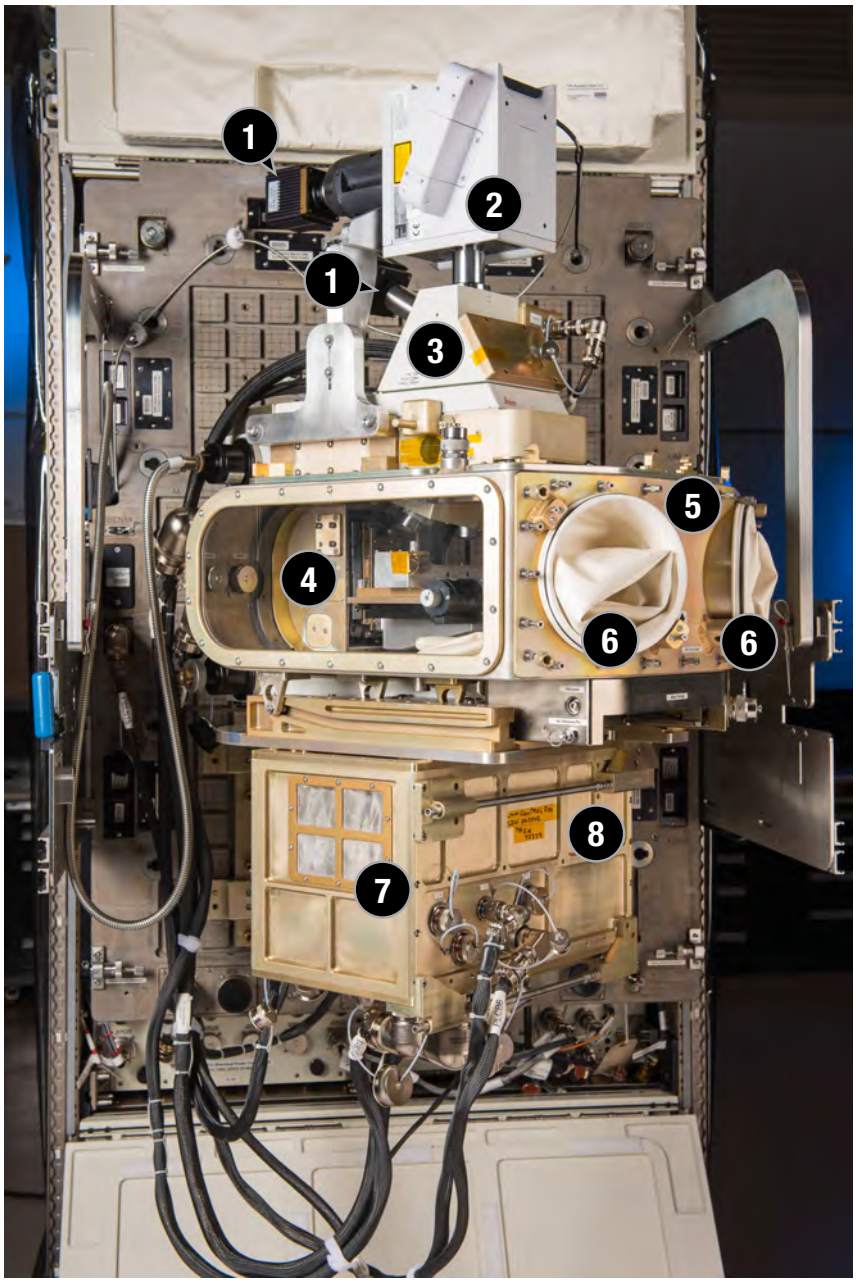
LMM Supported in the Fluids Integrated Rack

The LMM operates in the Fluids Integrated Rack (FIR), which is located in the U.S. Destiny Laboratory of the ISS. The FIR provides the LMM with the laboratory infrastructure common to most investigations, including an optics bench, temperature control, power control, illumination, imaging and frame capture, data processing, and other resources. The FIR also provides isolation from vibrations on the station to allow for a more stable environment to obtain high-resolution images. The LMM, in conjunction with the FIR, will help fulfill the vision of a true laboratory in space, which is ideal for low-cost payload development.

Critical Research Enabled by LMM

How matter is organized and moves on the microscopic level profoundly affects the macroscopic world. Understanding these processes will help scientists and engineers build more efficient machines and consumer products both on Earth and space applications. A suite of experiments is enabled by the LMM to allow for a detailed characterization of fluids, colloids, two-phase media, and biological samples. In the future, the LMM could be used to assist in maintenance of station crew health, to advance knowledge of the effects of space on biology, and to contribute to long-term mission space exploration.

1. **IMPERX GEV–B2020 cameras**
   —Available 2016
2. **Yokogawa CSU–X1 confocal scanner**
   —Available 2016
3. **Microscope**
4. **Auxiliary fluids container—side view**
5. **Auxiliary fluids container—front view**
6. **Glove ports**
7. **LMM control box**
8. **Equipment transfer module location**



Modified Microscope

Engineers at NASA Glenn Research Center modified a Leica RXA laboratory-grade microscope by adding 23 micromotors to permit remote control by scientists on the ground and to meet the demands of space flight and crew-tended operations. As such, it contains all of the necessary optical components for use as a fully functional microscope. The microscope can house many different lenses corresponding to magnifications of 2.5×, 4×, 10×, 20×, 40×, 50×, 63× (air), 63× and 100× oil-coupled objectives. Present capabilities include brightfield and epi-illumination microscopy. Future planned capabilities include high-resolution color video microscopy, condenser assembly, confocal microscopy, and possibly laser tweezers.

A modified Leica RXA research imaging light microscope with powerful laser-diagnostic hardware and interfaces.

LMM Control Box (LCB)

The electrical design of the LMM uses parts of the existing electronics of the Leica microscope and supplementary internal and external electronics that support enhanced automation and imaging capabilities. Motors and linear actuators have been added to motorize the manual functions of the Leica microscope. The LCB provides 16 axes of control for stepper motors and 4 axes of control for servo motors.

The LMM control box contains the control avionics for the entire LMM subrack facility.

Q-Imaging Retiga 1300 camera.

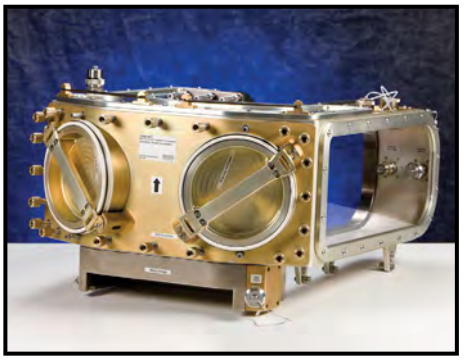
IMPERX GEV–B2020.

Imaging Cameras

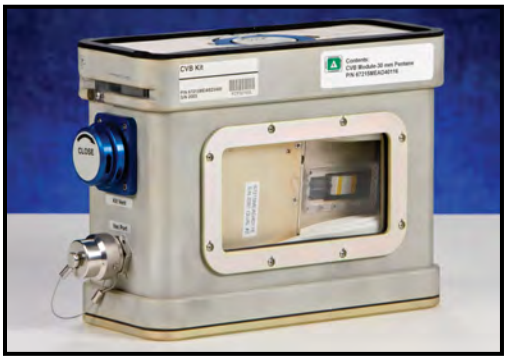
Two cameras can be mounted on the headpiece of the microscope; one coaxially with the viewing axis of the microscope and one mounted at an angle on the confocal tube assembly. The two present cameras employed are identical Q-Imaging Retiga 1300 units. In addition to these two cameras, there is a small surveillance camera that can be mounted inside the AFC (shown on the next page). The surveillance camera has a fixed window size of 640×480 pixels/frame. The Q-Imaging 1300 cooled monochrome camera (6.7×6.7 μm) has a maximum window size of 1280×1024 pixels/frame. A camera upgrade was initiated in 2014 using the IMPERX GEV–B2020. The resolution of this camera is 2048 × 2048 pixels/frame.

Auxiliary Fluids Container (AFC)

The LMM provides an enclosed work area called the AFC, which is the main work area for sample cell processing and containment for fluids and shatterable materials. The AFC consists of two sealed glove ports, gloves, and an attachment port for the equipment transfer module (ETM) used for transporting experiment samples from stowage to the LMM. The AFC is fastened to the microscope body and sealed to provide a clean working space and one level of containment. Glove ports allow access to the sample area for cleaning before opening the box and experiment sample changeout or reconfiguration. The ETM can be configured to support various experiment modules and is located below the AFC, which has a pass-through for the samples. Materials are thus transferred without the risk of contamination release. The ETM is loaded with experiment modules on the ground and provides contained storage until the samples are utilized in the experiment.



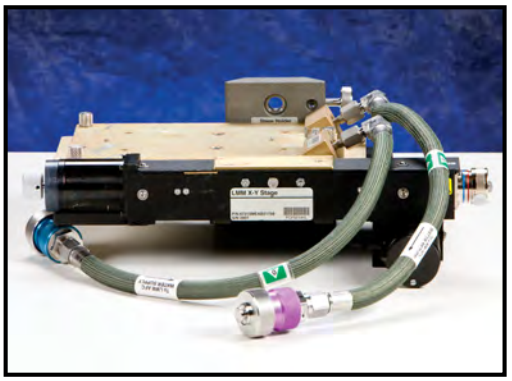
Auxiliary fluids container.



Experiment transfer module.

X-Y Stage

During all testing in the AFC, the experiment sample is always moved relative to the "fixed" objective lens of the microscope. Movement is accomplished by first mounting the sample on the translation stage assembly, which automates the movements in the plane normal to the main axis of the microscope (i.e., in the X-Y plane). Movement along the main axis of the microscope (Z direction) is accomplished through the stage mount, which is connected to a Z-drive mechanism in the body of the microscope. Translation in the X-Y plane is done using motors on the translation stage itself. These motors receive drive signals through the single electrical fitting located at the end of the X-axis housing.



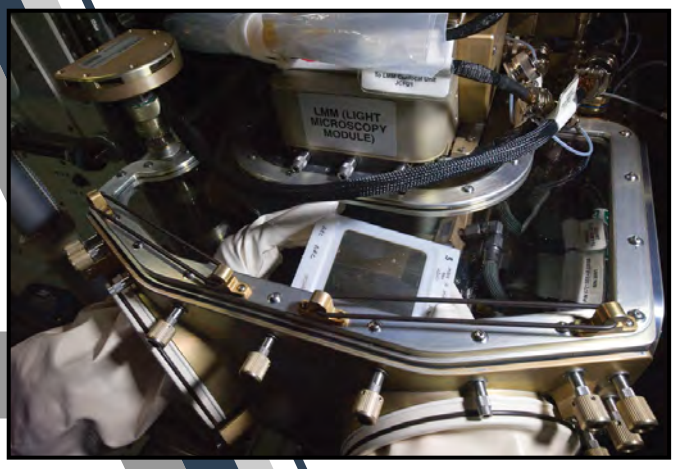
X-Y stage.

Test Cells

The LMM has the ability to image custom made microscopic slides for biological tests and sample wells containing colloids, along with commercial OptiCells™, which are a unique cell culture format for growing, monitoring, and transporting biological cells. OptiCells™ contain two parallel, gas-permeable, cell culture treated, polystyrene membranes attached to a standard microliter plate-sized frame. Each side has a growth area of 50 cm^2, total 100 cm^2, 75-µm thick membranes, 2 mm apart. A standard petri dish can be used for plant biology investigations.

Biological Sample Cells

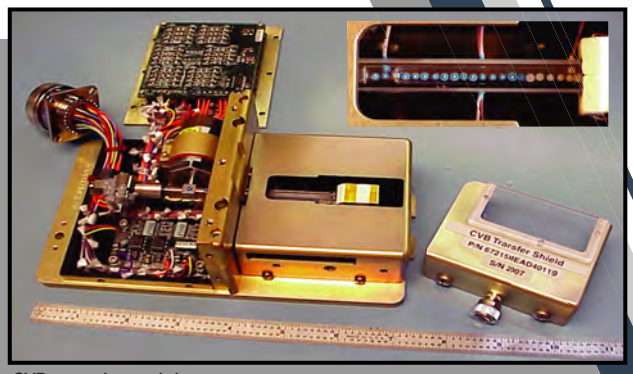
Biological samples for the LMM launched on the Space Shuttle Discovery's STS–133 mission on February 24, 2011, included fixed slides containing yeast, bacteria, a leaf, a fly (Drosophila), a butterfly wing, tissue sections and blood, and six containers of live *C. elegans* worms. The wing was from "Butterflies in Space" (a previous study that involved students from around the country) that was flown into space in 2009 on STS–129. In addition, some of the worms were descendants of those that survived the Space Shuttle Columbia STS–107 accident. These experiments were operated using OptiCells™ (right) and sample slides (see previous page).



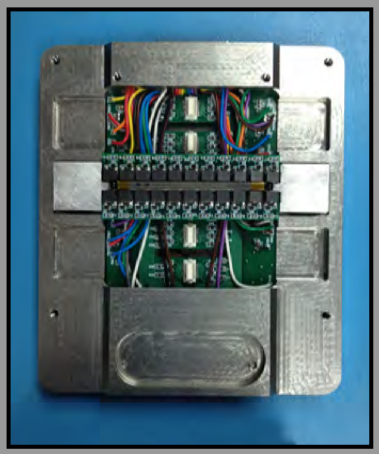
OptiCells™ being loaded into LMM.

Physical Science Sample Cells

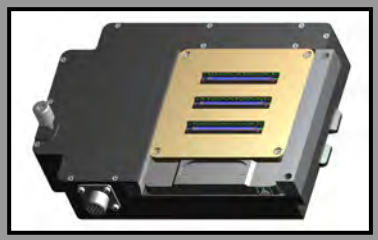
On-going experiments in thermophysics are conducted in the Constrained Vapor Bubble (CVB) sample modules. In development are "specialty cells" that use physical properties (temperature, E-field) to implement specific colloidal science test matrices. The colloidal science specialty cells will have a geometry and complexity similar to that of the CVB sample module. Prototype temperature, electric field, and biology specialty cells are in development.



CVB sample module.



Breadboard-engineering model ACE–T.



Computer rendering of ACE–T assembly.

Pre-Advanced Colloids Experiment (PACE) LED Base

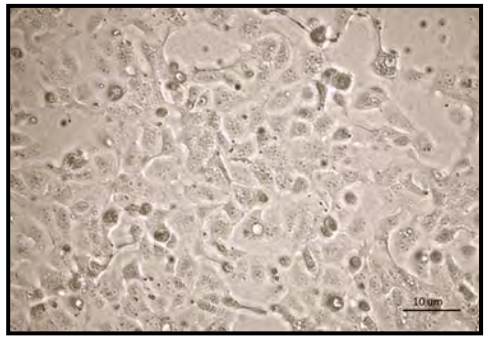
Present operations use a sample module mounted on a PACE light-emitting diode (LED) base. The ACE-M sample module has 15 small wells (1.2 µl) to observe colloid experiments in epi-illumination. The current experiment with Procter & Gamble will test product stability. A planned experiment with the University of Pennsylvania will test temperature sensitive particles. The PACE module will be modified to include a heater for this experiment. ACE–T uses the same mechanical interface as the PACE LED base. When ACE–T is launched in 2015 it will become the new sample module.

In Situ Mixing (2015)

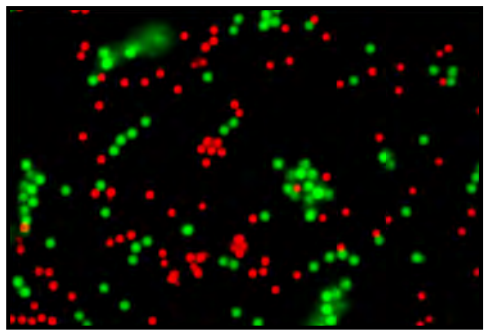
In situ mixing allows for the observation of samples shortly after mixing and for samples to be reinitialized at any time without intervention by the ISS crew. In situ mixing is a feature of the ACE–T specialty cell.

Confocal Microscopy (2016—2020)

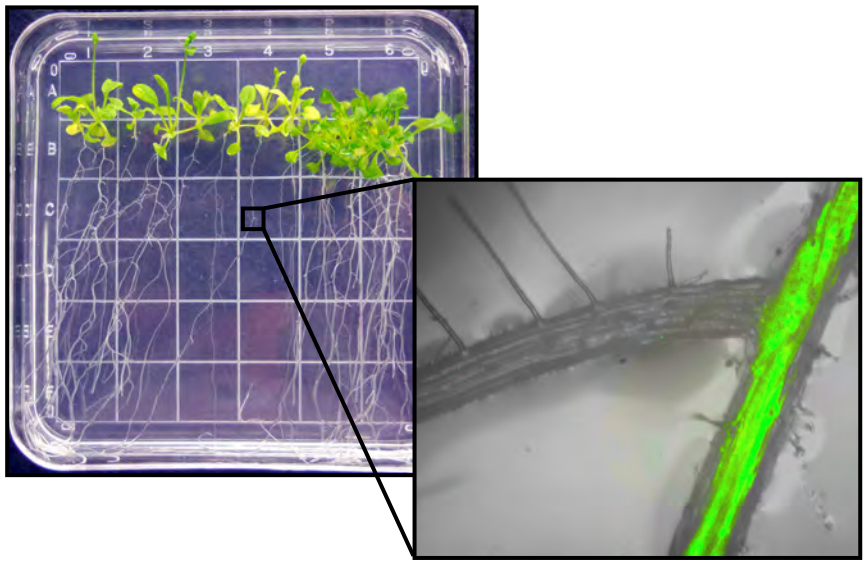
Confocal microscopy will be implemented using a 532-nm frequency- doubled Nd:YAG laser, a Nipkow disk confocal scanner, and an 8- to 12-bit digital camera. The scanner will allow up to 24 frames per second of confocal images to be collected by the camera. The crystal's three-dimensional structure will be reconstructed by assembling the image slices with an image analysis program from which colloidal growth, structure, and dynamics can be determined. The confocal module will be attached and aligned to the side of the LMM and will access the sample through a camera port on the Leica RXA. The microscope's reflected light turret will contain a reflecting mirror to direct the light to and from the sample. The confocal upgrade includes a custom image processor, two cameras, and a confocal scanner as shown on the cover.



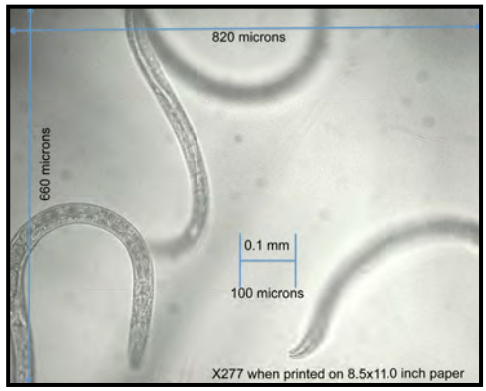
Cell culture sample imaged with LMM using 10× magnification. These cells were grown in an OptiCell™ on the ISS.



Confocal image from ground testing at 40× of multicolor 1.8 µm particles.



Fluorescent image of Arabidopsis (root) taken during LMM ground testing for CARA/Petri Plant demonstration planned for SpaceX-3 mission.



Epi-illuminated image of live C. elegans using a 10× objective. The C. elegans were cultured in an OptiCell™ on the ISS.

Laser Tweezers (Proposed)

Laser tweezers would be implemented using a system based upon a laser, beam-focusing optics, and holographic optical elements (HOEs) to steer the trap within the field of view of the microscope. Laser tweezers enable the trapping of a colloidal particle using radiation pressure by focusing a laser beam through a high-numerical aperture lens and manipulating the particle. Laser tweezers would be used to position particles and to measure the viscosity and viscoelasticity of the fluid. A particle can be trapped and oscillated at a fixed frequency. When this is done, the centroid of the trap and particle will not coincide; the difference in the two positions through the scan is a way to determine the driving force. Using this information, along with the motion, both linear and nonlinear viscoelastic properties can be computed.

LMM on the ISS.

For more information about the Light Microscopy Module please visit
http://spaceflightsystems.grc.nasa.gov/SOPO/ICHO/IRP/FCF/Investigations/LMM/

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